Short Communication

Broader T Cell Responses Directed Against Human Immunodeficiency Virus Type 1 in Infected Chinese Individuals Through Blood-Borne Transmission in Comparison with Mucosal Transmission

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Abstract

Cellular immune responses play a critical role in the control of human immunodeficiency virus type 1 (HIV-1), but less is known about the impact of transmission routes on immune defenses against HIV-1. Here, we report that subjects infected with HIV-1 through contaminated blood showed stronger HIV-specific T cell responses than those infected through mucosa, both in breadth (6.9 ± 2.5 vs. 2.3 ± 0.5 , $p = 0.0293$) and in magnitude [1270.0 ± 544.9 vs. 409.5 ± 121.3 SFU per million peripheral blood mononuclear cells (PBMCs), $p = 0.0223$], by using a matrix of 404 overlapping peptides spanning all expressed HIV-1 proteins in an interferon (IFN)- γ enzyme-linked immunospot (ELISpot) assay. Our observation indicates that different mechanisms might be involved in the priming/generating of anti-HIV-specific T cell responses through different transmission routes.

HIV-1-SPECIFIC T CELLS ARE KNOWN to play an important role in controlling the virus, and viral mutations associated with T cell pressure are frequently seen in association with disease progression.¹ Little is known about the quantitative and qualitative differences in HIV-1-specific T cell response between different transmission routes, especially blood-borne transmission among IV drug users and former plasma donors and sexual mucosal transmissions. Epidemiological observations indicate the relative inefficiency of the virus through sexual transmission,^{2,3} as recent findings showing low numbers of transmitted viruses through sexual infection suggest.⁴ Thus, the discrepancy of transmission efficiency between sexual and blood-borne transmission suggests potentially different HIV-1-specific T cell responses for each pathway. In this study, we investigated HIV-1-specific T cell responses in a group of former plasma donors (FPDs) who became HIV-1 infected through contaminated blood and IV drug users through sharing syringes compared with a group infected through sexual transmission.

Blood samples from 32 chronic HIV-1-infected Chinese Han individuals [all on highly active antiretroviral therapy (HAART)] were obtained and investigated in this study, among whom eight patients were infected by contaminated

blood (six FPDs and two intravenous drug users) and 24 were infected through sexual transmission (heterosexual and homosexual). Fresh peripheral blood mononuclear cells (PBMCs) were separated from whole blood using Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation and were stored in vapor-phase liquid nitrogen until use. Study subjects were recruited from the Beijing Youan Hospital. Relevant clinical data of all subjects are summarized in Table 1. The study was approved by the respective Institutional Review Boards of Beijing YouAn Hospital [IRB number 00004929], and all subjects gave written informed consent.

A total of 404 peptides, overlapping by 10 amino acids, most of which were 18 amino acids in length and spanned the entire expressed HIV-1 clade B genome (Gag, Pol, Vif, Vpr, Vpu, Rev, Tat, Env, and Nef), and 124 matrix peptide pools were modeled as described previously,⁵ with each of the 404 peptides represented in three different pools (three-dimension or three-dimensional peptide matrix) and with every pool including 8 to 11 different peptides. All peptides and peptide pools were then frozen at -80°C until use. ELISpot assays were performed as described previously.⁶ We used ELISpot for the Human Interferon- γ kit (Mabtech, Sweden) and

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TABLE 1. CLINICAL INFORMATION OF HIV/AIDS PATIENTS IN THIS STUDY

Subject	Gender	Risk factor	HIV RNA (copies/ml) ^a	CD4 ⁺ cell counts (cells/mm ³) ^b	Duration of diagnosis (m) ^b	Timing of HAART initiation (m) ^c	Duration of HAART (m)
CHR1	F	FPD	< LDL	501	168	104	62
CHR2	F	FPD	< LDL	356	168	126	39
CHR3	F	FPD	< LDL	335	57	7	43
CHR4	M	FPD	< LDL	376	35	1	30
CHR5 ^d	M	FPD	82,372	104	85	11	12
CHR6	F	FPD	390	589	104	30	74
CHR7	F	Ivdu	< LDL	508	93	59	31
CHR8	M	Ivdu	< LDL	446	74	0	74
CHR9	F	Hetero	< LDL	595	82	7	73
CHR10	M	Hetero	< LDL	557	171	99	69
CHR11	M	Hetero	310	397	56	18	38
CHR12 ^d	F	Hetero	2,200	246	134	12	18
CHR13	F	Hetero	< LDL	427	75	37	35
CHR14	M	Hetero	910	383	65	14	48
CHR15	F	Hetero	910	372	62	32	30
CHR16	M	Hetero	< LDL	317	78	47	31
CHR17	M	MSM	< LDL	412	60	22	38
CHR18	M	MSM	< LDL	609	57	0	57
CHR19	M	MSM	160	635	60	26	34
CHR20 ^d	M	MSM	Missing	201	72	47	13
CHR21	M	MSM	1,500	302	48	13	35
CHR22	M	MSM	< LDL	415	148	98	44
CHR23	M	MSM	< LDL	321	56	22	34
CHR24	M	MSM	< LDL	478	42	7	35
CHR25	M	MSM	< LDL	403	54	20	34
CHR26	M	MSM	< LDL	440	60	18	42
CHR27	M	MSM	< LDL	369	146	76	70
CHR28	M	MSM	< LDL	916	112	27	85
CHR29	M	MSM	< LDL	253	48	22	26
CHR30	M	MSM	< LDL	620	60	29	31
CHR31	F	MSM	< LDL	311	35	1	34
CHR32 ^d	M	MSM	Missing	152	164	120	12

^aMeasured at time of HIV-1-specific T cell analysis.

^bBetween time of diagnosis of HIV/AIDS infection and time of HIV-1-specific T cell analysis.

^cBetween time of diagnosis of HIV/AIDS infection and initiation of HAART treatment.

^dWho had undergone several treatment interruptions.

CHR, chronic; M, male; F, female; MSM, men who have sex with men; Hetero, heterosexual contact; FPD, former plasma donors; Ivdu, IV drug use; HAART, highly active antiretroviral therapy; LDL, lower than detectable levels.

96-well Multiscreen HTS IP plates (MSIP4510; Millipore, United Kingdom) in this section. The PBMCs were plated at a 0.5×10^5 to 1×10^5 viable PBMC per well and the final concentration of the peptides in the well was 2 μ g/ml. The spots were developed with AP conjugate substrate kit (Bio-Rad Laboratories; Hercules, CA). Plate results were read using a ChamSpot III ELISpot Reader System (Sage Creation, Beijing, China) and the number of specific T cells was calculated by subtracting the negative control values. The ELISpot data are expressed as the number of spot-forming units (SFU) per million PBMCs. Responses were regarded as positive if they had at least three times the mean number of SFU in six control wells and had to be >50 SFU/ 10^6 PBMCs.

Data are presented as mean $\pm$ standard error of the mean. Statistical analysis and graphical presentation were performed using SPSS 13.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 5.0. The data were analyzed using the Student's *t*-test with significance at $p < 0.05$.

Using the afore-mentioned three-dimensional peptide matrix approach with ELISpot assays, we screened a total of 32 HIV-1-infected individuals for T cell responses against the

entire expressed HIV-1 consensus clade B genome on a single peptide level. Through analysis of the data, we found that the majority of the HIV-1 protein subunits are targeted by virus-specific T cell responses, and the overall peptide recognition within all studied subjects is dominant in Nef, Vpu, and Gag proteins, as reported previously.⁷ We have identified 67 individual overlapping peptides given responses at least once over the total 404 peptides (67/404, 16.6%).

The specific T cell responses within individual subjects are depicted in Fig. 1A in breadth and total magnitude, respectively. Variables listed in Table 1 were demonstrated to be evenly distributed between the blood-borne and mucosa transmission groups, specifically 98.0 ± 17.0 vs. 81.0 ± 8.3 (m), $p = 0.249$ for duration of diagnosis, 42.3 ± 17.4 vs. 33.9 ± 6.6 (m), $p = 0.879$ for timing of HAART initiation, 45.6 ± 7.9 vs. 40.3 ± 3.8 (m), $p = 0.557$ for duration of HAART, and 401.9 ± 52.5 vs. 422.1 ± 34.4 (cells/mm³), $p = 0.948$ for CD4 count. Even though there were no significant differences in the clinical data among our studied subjects between transmitted routes, former plasma donors together with IV drug users presented a stronger HIV-1-specific T cell response in

FIG. 1. Comparison of breadth and magnitude of HIV-1-specific T cell responses between the HIV-1-infected study subgroups infected through contaminated blood and through sexually related mucosal transmissions. **(A)** The overall T cell responses within individual subjects are illustrated by the number of responsive individual peptides for each protein subunit and the total magnitude of T cell responses for each subject. **(B)** The breadth of HIV-1-specific T cell responses was expressed as the number of responsive overlapping peptides on the y-axis. **(C)** The total magnitude of T cell responses to overlapping peptides by each responder within each group was expressed in SFU/10⁶ peripheral blood mononuclear cells (PBMCs) on the y-axis. Each bar represented mean $\pm$ SEM; *p* values were calculated using the Mann-Whitney test. (Continued $\rightarrow$)

total. The HIV-1-infected subgroup infected through contaminated blood recognized more overlapping peptides than mucosa, expressed through the number of responsive overlapping peptides (6.9 ± 2.5 vs. 2.3 ± 0.5 , $p = 0.0293$, Fig. 1B). Also, the HIV-1-infected subgroup through contaminated blood gave stronger specific T cell responses based on the total magnitude of response within each patient (1270.0 ± 544.9 vs. 409.5 ± 121.3 SFU per million PBMCs, $p = 0.0223$, Fig. 1C).

In this study, we have shown that HIV-1-infected subjects infected through contaminated blood give a broader HIV-1-

specific T cell response with higher magnitude in comparison with mucosal transmission through sexual relations, in which we have excluded variables (importantly as duration of diagnosis, timing of HAART initiation) that could account for the observed difference in interferon (IFN)- γ response showing the groups are comparable based on the data provided. Many host and virological factors could determine the quantity and quality of the overall T cell responses such as individual genetic background, infecting virus, and infection routes.

FIG. 1. (Continued).

Researchers have found only single or very limited numbers of transmitted viruses during acute HIV-1 infection in most patients infected through sexual transmission.⁴ Indeed, around 80% of cases of heterosexual transmission have shown that a single virus establishes infection,^{4,8} which is consistent with the epidemiological observation of the laggard efficiency of HIV-1 transmission through most sexual routes.^{2,3} In the present study, 32 HIV-1-infected chronic individuals infected from different route were studied. We found that T cell responses had limited breadth and magnitude among subjects infected through sexual routes (homosexual and heterosexual), compared to subjects infected through contaminated blood. The lower HIV-1-specific T cell responses could be due to the single or narrowed transmitted virus during acute HIV-1 infection.

The route of infection and microenvironment for antigen delivery have been shown to have an important impact on the magnitude and quality of the T cell responses in an Adenovirus-Based HIV-1 Vaccine study, most likely due to the differences in the capacity of T cell priming⁹; low-frequency, monofunctional CD8 T lymphocytes with restricted anatomic trafficking patterns were observed in mucosal routing of vaccination, which is in agreement with our observation concerning the lower magnitude and narrower T cell responses in patients infected through sexual transmission when compared with blood-borne transmission. The mechanisms that underlie this, such as antigen presentation and T cell priming in the local microenvironment, merit further investigation. Ideally it would have been preferable to study untreated patients with similar infection times and viral loads, but such a cohort was inaccessible for this study, particularly in the identification of untreated patients with blood-borne transmission.

Irrespective of these considerations, we believe that there is still an advantage in comparing the patients with HAART treatment, as it is known that HAART treatment would affect the strength of the T cell responses, mainly due to reduced viral load, therefore reducing the antigen stimulation required for T cell expansion.^{10–12} In our case, most (almost all) patients (both infected through contaminated blood and sexual relations) are on long-term treatment, with either a low or undetectable viral load, and therefore the amount of antigen for T cell stimulation/expansion in the blood should be comparable between the two groups. This suggests that the differ-

ences we saw between the two groups could not be due to treatment but more likely represent real differences due to the nature of the transmission route.

In conclusion, this study provides a comprehensive analysis of the profile of HIV-1-specific T cell responses in a group of Chinese Han subjects infected with HIV-1 through different infection routes and has observed broader and stronger HIV-1-specific T cell responses in subjects infected through contaminated blood rather than through sexual transmission.

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Author Disclosure Statement

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